

THE CATHOLIC UNIVERSITY OF AMERICA

BIOLOGICAL STUDIES No. 9

**Effects Of Thyroid Inhibitors Upon Tail
Regeneration In The Tadpole**

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF ARTS AND
SCIENCES OF THE CATHOLIC UNIVERSITY OF AMERICA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

BY

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College of New Rochelle



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EFFECTS OF THYROID INHIBITORS
UPON TAIL REGENERATION IN THE TADPOLE

This dissertation was conducted under the direction of Dr. William Gardner Lynn as Major Professor, and was approved by Dr. Edward G. Reinhard and Reverend Henry Wachowski as readers.

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EFFECTS OF THYROID INHIBITORS UPON TAIL
REGENERATION IN THE TADPOLE

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EFFECTS OF THYROID INHIBITORS UPON TAIL REGENERATION IN THE TADPOLE¹

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A. INTRODUCTION

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The rapidity and completeness with which regeneration of the tail occurs in amphibian larvae has made these animals favored objects for the investigation of regenerative processes. Zeleny (16) carried out a detailed study of tail regeneration in *Rana clamitans* tadpoles and distinguished three successive phases in the process. Immediately following amputation there is a period of "regulation" characterized by cell migration and reorganization at the cut surface. Following this comes a phase of proliferation during which the regenerating area rapidly increases in size. This is succeeded by a period of cell and tissue differentiation which is accompanied by a gradual decline in the rate of growth of the regenerating region. The time required for these phases, other factors being constant, varies with the extent of the amputation. When one-half of the tail is removed the regulative phase occupies two to four days, the proliferative phase lasts three to six days and the differentiative phase continues throughout the rest of the regenerative period.

Speidel (29) found that the course of regeneration is definitely affected by treatment with thyroid extract and, moreover, that the effect differs depending upon the phase of regeneration during which the treatment is instituted. If thyroid administration is begun shortly before or coincident with amputation of the tail, the regulative phase

¹A contribution from the Department of Biology, The Catholic University of America, Washington, D. C. This paper is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express her deep appreciation to Professor William Gardner Lynn, who suggested this problem to her, for his many helpful suggestions both during the course of the investigation and in the preparation of the manuscript. To Professor Edward G. Reinhard and Reverend Father Henry Wachowski sincere thanks are due for their helpful suggestions.

is interfered with and almost no regeneration occurs. If, on the other hand, administration of the extract is deferred until near the end of the regulative phase or later, growth and proliferation are accelerated and the onset of the differentiative phase is hastened. Thus in the first case the hyperthyroid condition results in inhibition of regeneration, while in the second it causes an increase in the growth rate and a rapid maturing of the tissues. Speidel's experiments with tadpoles of the wood frog (*Rana sylvatica*), the bullfrog (*Rana catesbiana*), the tree frog (*Hyla crucifer*), and the toad (*Bufo americanus*) showed that the general effect of thyroid extract upon regeneration is similar in all of these forms.

In view of these results it would be of interest to follow the course of regeneration in tadpoles in which the thyroid gland is absent or non-functional. A search of the literature reveals almost no information on this subject. There appears to be no comprehensive study of regeneration in thyroidectomized or hypophysectomized animals despite the fact that the techniques for performing these operations in amphibian larvae are well known and quite simple.

The present investigation is an attempt to approach this problem, not by operative techniques, but by use of some of the newly developed chemical inhibitors of the thyroid. The independent discovery by Richter and Clisby (42), Kennedy (42), and Mackenzie and Mackenzie (43) of the goiterogenic activity of the thioureas has led to extensive study of these drugs. It has been shown that in mammals administration of these substances causes hypertrophy of the thyroid epithelium and reduction in the amount of colloid with accompanying symptoms of hypothyroidism.

Gordon, Goldsmith, and Charipper (43, 45) were the first to report experiments dealing with the effects of thiourea upon the amphibian larva. They found that *Rana pipiens* tadpoles raised in a .033 per cent solution of the drug from the stage of first appearance of the hind-limb buds failed to metamorphose, having hind limbs only three to five millimeters long at the time when the controls had completely transformed into frogs. Hughes and Astwood (44) obtained similar results using a related compound, thiouracil, in a concentration of 1:2000. Since simultaneous administration of thyroxin to these animals promptly induced metamorphosis it seems clear that the thiouracil does not act directly upon the tissues but brings about its

effect through interference with the normal production of the thyroid hormone. Still another compound, phenylthiourea, in a concentration of .005 per cent has been found by Lynn (47) to be an effective inhibitor of the thyroid function in the anuran *Eleutherodactylus ricordii*.

In the present study two of these drugs, thiourea and phenylthiourea, have been used as thyroid inhibitors and tail regeneration under various conditions of treatment has been investigated.

B. MATERIALS AND METHODS

Tadpoles of the bullfrog (*Rana catesbiana*) were obtained from a pond at McDonogh, Maryland. Large numbers were available, making it possible to select specimens of uniform size. Tadpoles of the leopard frog (*Rana pipiens*) were obtained by pituitary-induced ovulation and artificial insemination using adults obtained from a dealer in New York State. For each experiment tadpoles from a single clutch of eggs were used and were carefully selected for uniformity of size.

The bullfrog tadpoles were kept individually in glass jars each containing one liter of tap water. The only exception to this was in the first experiment to be reported, in which five tadpoles were kept in each jar. The leopard frog larvae were raised in white enameled pans measuring 9 x 15 x 2 inches. Each pan contained 10 tadpoles in one liter of culture medium. Animals were fed principally upon pabulum and strained liver-soup preparation. All cultures had excess food available and uniform conditions of light and temperature were maintained. The culture medium was changed daily except in cases specifically noted in the accounts of individual experiments.

Tail amputation was carried out under anesthesia with MS-222 in concentration of 1:4500. In all experiments the distal two-thirds of the tail was removed. Measurements of body dimensions and length of regenerating tail were taken by means of dividers under a binocular microscope. At various stages in the course of the experiments, tadpoles were preserved in 10 per cent formalin for histological study. Materials for sectioning were dehydrated in cellosolve, cleared in xylol, embedded in paraffin, sectioned at 10 μ and stained with Mallory's triple stain or Harris' hematoxylin and eosin.

C. RESULTS

1. Effects of thiourea upon regeneration when administered during the proliferative phase.

Speidel's (29) experiments demonstrated that the accelerating effect of thyroid substance upon tail regeneration is most pronounced when treatment is instituted during the proliferative phase. It therefore seemed best to begin the present investigation with a study of the results of thyroid inhibition during this phase. This experiment also served as an exploratory investigation of the effects of various concentrations of thiourea and the relative effectiveness of administration by injection, as compared with administration by solution of the drug in the culture medium.

Thirty-five bullfrog tadpoles ranging from 68 to 72 mm. in total length were divided into seven groups of five. Each group was kept in a large glass container holding two liters of culture medium. The distal two-thirds of the tail was removed from all animals on the same day. Regeneration was allowed to proceed without treatment for six days at which time the process was in the early proliferative phase. The various groups were then subjected to different treatment as follows:

- Group 1:* given daily intra-peritoneal injections of .1 cc of 0.1 per cent thiourea
- Group 2:* given daily intra-peritoneal injections of .1 cc of 0.05 per cent thiourea
- Group 3:* given daily intra-peritoneal injections of .1 cc of 0.005 per cent thiourea
- Group 4:* kept in culture medium consisting of a solution of .05 per cent thiourea in tap water.
- Group 5:* kept in culture medium consisting of a solution of .01 per cent thiourea in tap water.
- Group 6:* controls, given daily injections of .1 cc of distilled water.
- Group 7:* controls, kept in tap water.

Culture media were changed and injections were given daily for a period of 27 days. Measurements of the regenerating tails were made daily and the results are given in the accompanying table and graph (table 1 and figure 1). Since the individual measurements within a group never varied more than three millimeters, only the averages are given here. Also, since both groups of controls ran exactly parallel,

TABLE 1
EFFECTS OF THIOUREA UPON TAIL REGENERATION, WHEN ADMINISTERED DURING THE
PROLIFERATIVE PHASE

Day of Regeneration	Average length of regenerating tail in mm.					Controls
	Thiourea injections 0.1%	0.05%	0.005%	Thiourea solutions 0.05%	0.01%	
6	2	2	2	2	2	2
7	2	2	2	2	2	2
8	2	3	3	3	2	2
9	3	3	3	3	3	3
10	3	4	4	4	4	4
11	4	4	5	4	5	5
12	5	5	6	4	6	6
13	6	6	7	5	7	7
14	6	6	7	5	8	7
15	7	6	7	6	8	8
16	7	6	7	7	8	9
17	7	6	7	7	9	10
18	7	7	7	8	9	10
19	7	7	7	8	10	11
20	8	8	7	8	11	11
21	8	8	8	8	11	12
22	8	8	8	8	11	12
23	9	8	8	9	12	12
24	9	8	9	9	13	13
25	9	8	10	9	14	14
26	10	8	10	9	14	14
27	10	8	10	9	14	15
28	10	9	10	9	15	15
29	10	9	10	9	15	15
30	10	9	10	10	15	15
31	11	9	11	11	16	15
32	11	9	11	11	16	15

averages are given for the two groups. It must be mentioned that during the course of the experiment four animals were lost. Two died in the .05 per cent solution; one on the seventh day of treatment and one on the twenty-fourth day. Two died in the group injected with .1 per cent thiourea; one on the thirteenth day and one on the seventeenth day. No other animals showed any signs of ill effects throughout the experiment.

Examination of the graph shows clearly that treatment with thiourea causes a definite decrease in the rate of tail regeneration. All three of the groups in which administration was by injection showed a pronounced effect which first became apparent about nine days after the beginning of treatment (fifteenth day of regeneration). The strength of the dosage, within the limits tested, seems to bear no

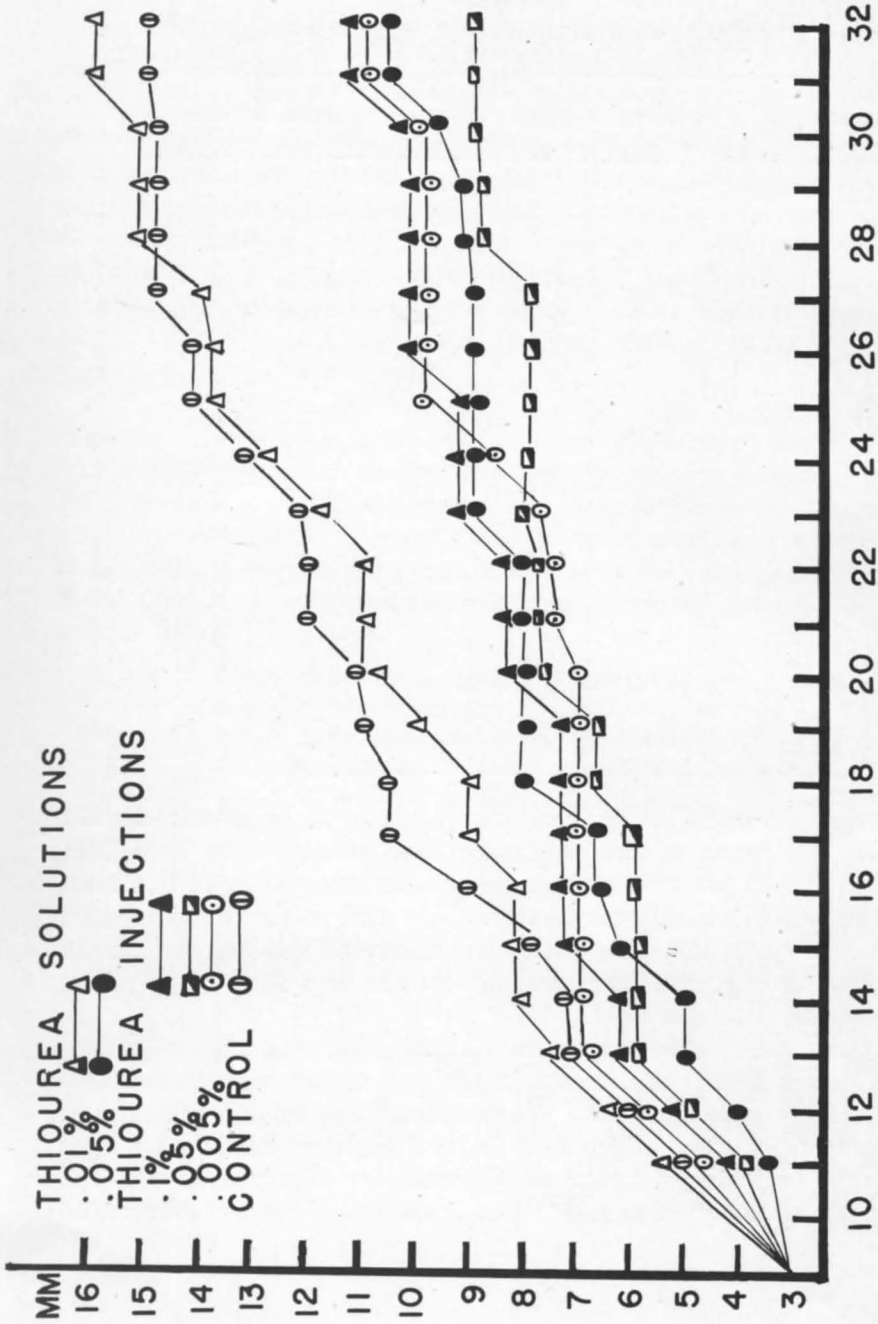


FIGURE 1
Effects of Thiourea upon regeneration when administered during the proliferative phase. (The abscissa is days of experiment, the ordinate is average length of regenerated tail in millimeters.)

relation to the intensity of the effect. This is what would be expected if all three dosage levels are sufficient to cause complete thyroid inhibition, and if the effect of the drug is assumed to be due to its influence on thyroxin-production rather than to any direct action on the regenerating tissues.

In the case of administration by addition of thiourea to the culture medium the situation is somewhat different. The curve for regeneration in .05 per cent thiourea shows an effect which parallels that produced by the various injection levels. On the other hand, the curve for regeneration in .01 per cent thiourea is not significantly different from that for the controls. This indicates that the latter concentration is too low to inhibit thyroid activity when administered in the culture medium and that the thyroxin level in tadpoles raised in this solution remains high enough to permit regeneration at the normal rate.

2. *Effects of thiourea upon regeneration when administered prior to, or coincident with, tail amputation.*

In view of Speidel's findings that thyroxin inhibits regeneration if administration is begun before or simultaneously with tail amputation, it now seemed desirable to study the course of regeneration in tadpoles in which thiourea treatment was instituted prior to amputation. Under such treatment the animals would presumably have a very low thyroxin level from the very beginning of the regeneration period. The experiment just reported indicates that an effective method of administration of the drug is injection at a concentration of .05 per cent and this method was therefore used exclusively in the present series.

Thirty-six bullfrog tadpoles ranging from 68 to 75 mm. in length were used. Because of the somewhat greater range in length as compared with the previous group it was decided to keep individual controls for each experimental animal. Eighteen pairs were therefore selected so that the two members of a pair differed by not more than two millimeters in total body length. One animal of each pair was given the injections while the other served as its control. Treatment was as follows:

Group A: Seven animals were given injections of .2 cc of .05 per cent thiourea on alternate days for 24 days. At the end of this time tail amputations were performed on these tadpoles

and their controls. The injections were continued throughout the course of the experiment and measurements were taken every other day from the fourth to the sixteenth day of regeneration.

Group B: Seven experimental animals were given injections of .2 cc of .05 per cent thiourea on alternate days for 16 days before amputation. Injections were continued and measurements taken throughout the first 16 days of regeneration.

Group C: Four experimental animals received their first injection on the same day that the tails were amputated. Injections were continued on alternate days for the first 16 days of regeneration as in the other groups.

The individual measurements of the regenerating tails are given for all of these animals and their controls in table 2. It will be seen that none of these treatments had any significant effect upon the early stages of regeneration. The regulative phase is neither retarded nor accelerated, experimental animals and controls regenerating at the same rate throughout the first 10 days of regeneration. After this a slowing down of the growth-rate of the tails of the injected tadpoles is shown in most of the pairs. This was, of course, to be expected since the injections were being continued and it has already been shown that treatments during the proliferative phase result in retardation of the regenerative rate. In the present series of experiments this effect is first apparent in most pairs on the 14th day of regeneration, which agrees closely with the time required for the effect to manifest itself in the first series reported.

3. Effects of thiourea and phenylthiourea upon tail regeneration during different periods of the larval history.

Zeleny (16) has noted that the normal rate of regeneration varies with the age of the larva. Since this can probably be attributed in part to the normal increase in activity of the thyroid gland in older tadpoles which are approaching metamorphosis, an experiment was now undertaken to test the effects of thyroid-inhibition upon regeneration in young and old larvae. *Rana pipiens* tadpoles were used for this study because their short larval period makes them more suitable than the bullfrog for such work. Since it was also desired to test the relative effects of thiourea and phenylthiourea, both of these

TABLE 2
EFFECTS OF THIOUREA UPON REGENERATION WHEN ADMINISTERED PRIOR TO,
OR COINCIDENT WITH, TAIL AMPUTATION

Group	Animal	Days of Regeneration						
		4	6	8	10	12	14	16
A	5 C	1	2	3	5	7	9	10
	5 I	1	2	3	5	7	8	9
	6 C	1	2	3	5	7	10	10
	6 I	1	2	3	4	6	7	8
	4 C	1	2	3	4	7	9	9
	4 I	1	2	3	5	7	8	8
	9 C	1	2	4	5	6	8	9
	9 I	1	2	4	5	6	6	7
	2 C	1	2	3	5	7	9	10
	2 I	1	2	3	4	6	9	10
	16 C	1	2	3	4	7	8	9
	16 I	1	2	3	5	6	7	8
	18 C	1	2	3	5	7	9	9
	18 I	1	2	3	4	5	6	6
B	15 C	1	2	3	5	6	7	9
	15 I	1	2	3	4	5	6	8
	1 C	1	2	4	6	8	9	10
	1 I	1	2	4	6	8	10	11
	3 C	1	2	3	5	7	7	9
	3 I	1	2	3	4	5	6	8
	12 C	1	2	4	4	7	8	10
	12 I	1	2	4	4	6	7	9
	10 C	1	2	4	5	6	7	8
	10 I	1	2	5	6	8	9	10
	13 C	1	2	3	5	7	8	9
	13 I	1	2	3	5	6	7	9
	14 C	1	2	3	5	6	7	8
	14 I	1	2	3	4	6	7	9
C	7 C	1	2	3	5	7	10	10
	7 I	1	2	3	4	6	8	8
	8 C	1	2	4	5	6	8	9
	8 I	1	2	4	4	6	7	8
	11 C	1	2	4	5	7	8	10
	11 I	1	2	4	4	6	9	10
	17 C	1	2	3	5	7	9	9
	17 I	1	2	3	4	6	8	9

C—control animal.

I—injected animal. Regenerated tail length in mm.

drugs were utilized. Phenylthiourea had already been found to be effective in inhibiting thyroid function when administered in the culture medium in the rather low concentration of .005 per cent. In view of this fact, and in consideration of the small size of *Rana pipiens* larvae, it was thought that thiourea might also be effective at

this concentration rather than at the higher ones which had proved necessary in the bullfrog. In this experiment, therefore, both drugs were administered by being added to the culture media in a concentration of .005 per cent.

Tadpoles hatched in the laboratory were raised in enamel pans, ten animals in each pan, until they reached the desired stages. The following experimental series were run:

Group A: Twelve cultures of larvae (120 animals) were raised in tap water until the tadpoles reached an average length of 24 mm. At this time they were 53 days of age. Four of the cultures were then transferred to .005 per cent thiourea, four were transferred to .005 per cent phenylthiourea and four were continued in tap water as controls. Amputation of the tail was carried out in all animals and the course of regeneration was followed by means of measurements made at varying intervals.

Group B: Twelve cultures were raised until the tadpoles reached an average length of 34 mm. (81 days). At this time the larvae all had well developed hind-limb buds 2 to 3 mm. long. Four cultures were then transferred to .005 per cent thiourea, four to .005 per cent phenylthiourea and four were used as controls. Tail amputation was performed and regeneration was followed as before.

TABLE 3
EFFECTS OF .005% THIOUREA AND .005% PHENYLTHIOUREA UPON TAIL REGENERATION
IN YOUNG AND OLDER LARVAE

	Day of Regeneration	Average length of regenerating tail in mm. Thiourea	Phenylthiourea	Controls
Group A	8	3	3	3
	10	3	3	4
	13	5	3	6
	19	9	5	9
	26	13	7	12
	38	15	10	14
Group B	7	3	2	3
	14	8	6	7
	21	11	7	10

The results of this experiment are given in table 3. It will be seen that in both Group A and Group B the animals raised in .005 per cent thiourea regenerated at essentially the same rate as did the controls.

Clearly this concentration is not high enough to be effective when added to the culture medium. On the other hand, .005 per cent phenylthiourea caused a retardation in both young and old larvae, the effect being first shown during the second week of regeneration. The primary purpose of this experiment was to ascertain whether there is any difference in the effect at early and late stages. There is no evidence that any such difference exists. It is to be noted, however, that the regeneration rates in the controls of Groups A and B did not differ significantly either, a fact which is not in agreement with previous observations on the relation between age and rate of tail regeneration.

To illustrate the results of this experiment, specimens from Group B were photographed and are shown in figures 2, 3, and 4. Figure 2 shows tadpoles on the seventh day of regeneration. The uppermost animal is a control, the middle animal is one treated with .005 per cent phenylthiourea and the lower specimen is from a .005 per cent thiourea culture. The regenerated portions of the tails of the control and thiourea-treated tadpoles both measure 3 mm., that of the phenylthiourea-treated animal measures 2 mm. Figure 3 shows specimens from the same three cultures on the fourteenth day of regeneration. The animals are arranged in the same order as in the preceding figure. The regenerated regions of the tails of control and thiourea-treated specimens now measure 8 mm.; that of the phenylthiourea-treated animal measures 6 mm. Figure 4 shows specimens on the twenty-first day, the tail measurements at this time being 13 mm. for the control and thiourea-treated tadpoles and 8 mm. for the phenylthiourea-treated specimens.

4. *Effects of thiourea upon tail regeneration after repeated amputation.*

The animals from Group A in the preceding experiment which had been operated upon at the 24 mm. stage had well-formed tails after 38 days of regeneration. It was decided that they could again be utilized, and all were therefore subjected to a second amputation. This second cut was made just proximal to the level of the first. At this time the animals were 91 days old. The control tadpoles and those in .005 per cent thiourea were beginning to show signs of the onset of metamorphosis, the hind limbs having reached an average

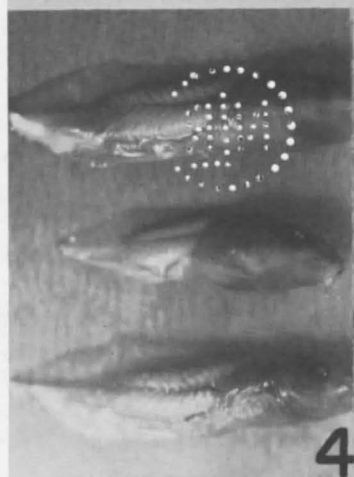


PLATE I
(Figures 2-7)

Photographs of experimental and control *Rana pipiens* tadpoles. x 1. In each figure the uppermost animal is the control, the middle animal was raised in .005 per cent phenylthiourea, and the lower animal was raised in .005 per cent thiourea.

2. Animals from Series 3, Group B on the 7th day of regeneration.
3. Animals from Series 3, Group B on the 14th day of regeneration.
4. Animals from Series 3, Group B on the 21st day of regeneration.
5. Animals from Series 4 on the 7th day of regeneration.
6. Animals from Series 4 on the 14th day of regeneration.
7. Animals from Series 4 on the 21st day of regeneration.

length of 6 mm. The tadpoles in .005 per cent phenylthiourea had hind limbs only 2 mm. long. Measurements of the regenerating tails of the tadpoles of Group A after the second amputation are given in

TABLE 4
EFFECTS OF .005% THIOUREA AND .005% PHENYLTHIOUREA UPON TAIL REGENERATION
AFTER SECOND AMPUTATION

Day of Regeneration	Average length of regenerating tail in mm.		
	Thiourea	Phenylthiourea	Controls
7	4	3	4
14	9	7	8
21	13	9	12
28	16	12	15

table 4 and specimens on the seventh, fourteenth, and twenty-first days of regeneration are illustrated in figures 5, 6, and 7. Again the animals in .005 per cent thiourea paralleled the controls while the tadpoles treated with .005 per cent phenylthiourea showed a retardation of regeneration rate. An unexpected result is to be observed by comparison of the figures in table 4 with those given for the correspondingly treated animals in table 3. It appears that in both control and experimental animals regeneration after a second amputation proceeds at a somewhat faster rate than does regeneration after first amputation.

In an attempt to obtain confirmation of this observation a further experiment was now performed. Thiourea alone was used but the concentration was increased to .033 per cent. The work of Gordon, Goldsmith, and Charipper (43) has shown that this concentration is sufficient to cause failure of metamorphosis in *Rana pipiens*.

Twenty tadpoles hatched in the laboratory were raised for 23 days. They were then placed in .033 per cent thiourea and maintained in this solution throughout the rest of the experiment. After 11 days treatment the first amputation was performed. The tadpoles averaged 25 mm. in length at this time. A second amputation was made 18 days after the first when the tadpoles were 52 days old and averaged 40 mm. in length. The third amputation was made 17 days later, the animals then being 69 days old. The average length at the time of the third amputation was only 42 mm., body growth having practically ceased at about 55 days.

Measurements of the regenerating tails were taken on alternate

days throughout the experiment. At the close of the experimental period the tadpoles were 86 days old and had been kept in .033 per cent thiourea for 63 days. None of them showed any signs of hind-limb growth. Many control animals from the same egg mass had completely metamorphosed by this time.

TABLE 5
EFFECTS OF THIOUREA UPON TAIL REGENERATION AFTER REPEATED AMPUTATIONS

Day of Regeneration	Average length of regenerated tail in mm.		
	1st amputation	2nd amputation	3rd amputation
4	1	2	3
6	3	4	5
8	4	6	6
10	7	8	7
12	8	9	9
14	10	10	10
16	11	10	10

The results of this experiment are given in table 5. It will be noted that the rate of growth of the tail is directly correlated with the number of amputations, being slowest after the first amputation and fastest after the third during the first week of regeneration. After the tenth day, however, the growth rates in all three groups run closely parallel.

5. *Histological observations.*

At intervals during the course of the various experiments described in this paper some individuals from each series were preserved. Later, selected specimens were sectioned to permit histological examination of their thyroids. This makes it possible to correlate the influence of the drugs upon tail regeneration with their effects upon the thyroid gland. As expected, it was found that animals which had received thiourea in such low concentrations that no effect upon regeneration could be detected had thyroids which were nearly normal in appearance. On the other hand, all animals whose regeneration had been definitely retarded by treatment with the drugs showed markedly affected thyroids.

The three photomicrographs shown in figures 8, 9, and 10 will suffice to illustrate these observations. The thyroid glands shown here are those of *Rana pipens* tadpoles used for the study outlined in section 3. Figure 8 shows the thyroid of an animal which had been

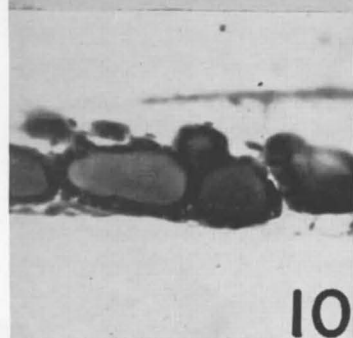
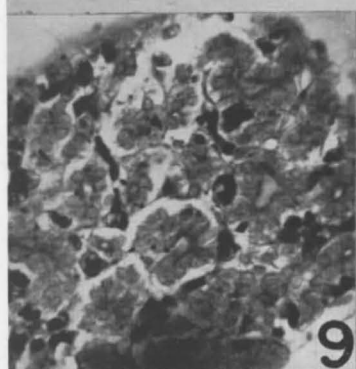
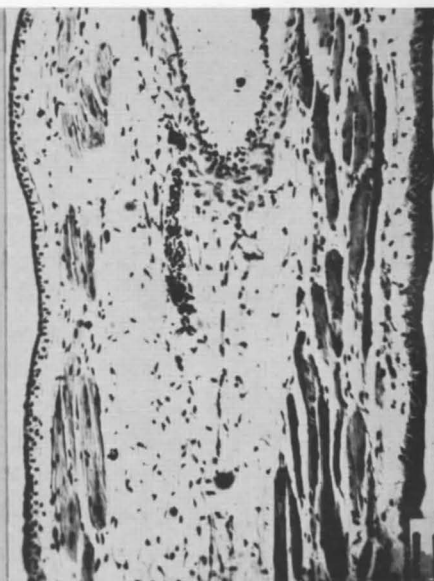
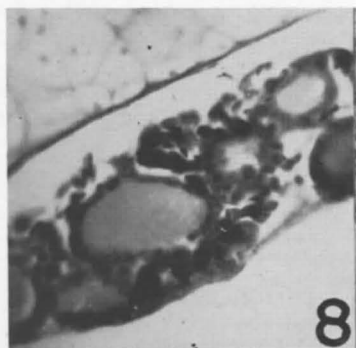


PLATE II

(Figures 8-12)

8. Section of thyroid gland of larva raised in .005 per cent thiourea solution for 75 days. x240.
9. Section of thyroid gland of control larva. x240.
10. Section of thyroid gland of larva raised in .005 per cent phenylthiourea for 75 days. x240.
11. Longitudinal section of the regenerating tail of a control larva on the 21st day of regeneration. x240.
12. Longitudinal section of the regenerating tail of a larva treated with .005 per cent phenylthiourea. Twenty-first day of regeneration. x240.

in .005 per cent thiourea for 75 days. Figure 9 shows the thyroid of an animal of the same age which had been in .005 per cent phenylthiourea for 75 days. Figure 10 shows the thyroid of a control of the same age. Obviously the phenylthiourea has caused a marked hypertrophy of the gland and an almost complete loss of colloid. The hyperemic condition of this thyroid is revealed by the many blood corpuscles scattered about between the follicles. The gland of the thiourea-treated tadpole differs but little from that of the control. There seems to be evidence of some increase in vascularity, but the follicular epithelium remains low and the amount of stored colloid present lies in the normal range. It will be remembered that the animals treated with .005 per cent thiourea in this experiment paralleled the controls in regeneration rate while those treated with .005 per cent phenylthiourea showed a retardation.

Some observations have also been made upon the histological features of the regenerating tail in experimental and control larvae. The general course of histo-differentiation in the control animals follows that described by previous authors (Allen 11, Naville 22). In experimental animals in which the regeneration has been markedly retarded there is good evidence that the retardation involves the process of differentiation as well as the rate of growth. Comparison of the regenerating tails of experimental animals with those of controls at different stages, shows that the time of appearance of myofibrillae and of notochord tissue is considerably later in the former than in the latter. This difference is illustrated in figures 11 and 12, which show longitudinal sections of the tails of *Rana pipiens* larvae on the twenty-first day of regeneration. The control animal (fig. 11) has well-differentiated muscles in the regenerated area while the experimental animal (fig. 12) has only a few myofibrillae at the proximal end of the new tail. Further study of this phase of the problem is needed, but it seems clear that thyroid-inhibition does affect the rate of differentiation in regenerating tissues.

D. DISCUSSION

The role of the thyroid secretion in anuran metamorphosis has been widely studied since the days of Gudernatsch's (12) pioneering experiments. It has been repeatedly demonstrated that removal of the thyroid anlage from the young larva results in complete failure to

metamorphose, while administration of the thyroid hormone to early tadpoles causes them to transform into frogs precociously. The phenomenon of metamorphosis is a complex one involving rapid growth and differentiation of some structures, such as the limbs, and resorption and ultimate disappearance of others, such as the gills and tail. It has been shown, however, that all of these changes are induced by the thyroid hormone, the differences in response in different tissues being due to the diverse constitutions of the tissues themselves.

Since the processes of growth and differentiation which occur during regeneration are in many respects similar to those of embryonic development, it was early suspected that they too might be affected by the thyroid secretion. Allen (18), in a brief abstract, recorded experiments upon tail regeneration in *Rana pipiens* tadpoles some of which had been thyroidectomized while others were fed thyroid extracts. He reported that in both of these groups, regeneration proceeded at the same rate as in untreated controls, and he therefore concluded that the thyroid gland does not influence the process of regeneration. As has been pointed out, however, Speidel's (29) very detailed experiments later demonstrated quite clearly that tail regeneration is affected by thyroid administration. Treatment during the early stages of regeneration causes inhibition of growth and differentiation; treatment during the later phases stimulates these processes.

In the present study the normal activity of the thyroid gland was reduced by administration of thyroid-inhibiting drugs to regenerating animals. It was found that if such inhibition is exerted during the early proliferative phase of regeneration the rate of growth of the regenerating tissue is definitely retarded. This is in complete agreement with Speidel's findings. On the other hand, treatment with thyroid inhibitors begun prior to or at the start of regeneration had no effect upon the early regenerative processes. Since Speidel had found that thyroxin treatment at this time caused a definite inhibition of regeneration, it might have been expected that decreasing the thyroxin level would give the opposite effect, namely acceleration of the regeneration rate. Since this is not the case it seems logical to conclude that the early phase of regeneration normally goes on independently of the thyroid gland but that it may be adversely affected by abnormally high thyroxin levels. If this hypothesis is correct, one would expect no effect upon this phase when the thyroid gland is removed or inhibited.

Zeleny (16) has reported that the rate of regeneration is somewhat faster in younger larvae than it is in older larvae. In the present study an experiment to test the effects of thyroid inhibition upon regeneration in *Rana pipiens* tadpoles at early and late larval stages failed to show any difference in regeneration rate at these times. It must, therefore, be concluded that the effect of thyroid hormone upon regeneration does not differ at different ages, at least within the limits tested. It is possible that experiments covering a wider interval and extending into actual metamorphic stages might reveal some differences in the effect.

Hoskins and Hoskins (19) in the course of a comprehensive study of the growth of various organs in thyroidectomized tadpoles of *Rana sylvatica* made incidental observations upon regeneration of the tail in a single thyroidectomized larva which was subjected to four successive amputations. They reported that the time required to regenerate the lost tissues increased steadily with each amputation. On the other hand, Zeleny (16) maintains that in normal tadpoles the regeneration rate is faster after a second amputation than it is after a first. In the present series of experiments, preliminary observations on tadpoles subjected to a second amputation seemed to indicate that regeneration does occur more rapidly under this condition, in both experimental and control animals. A further study of this matter was then carried out with thiourea-treated animals subjected to three successive tail amputations. The results show that, even under conditions of thyroid inhibition, the regeneration rate does increase after repeated amputations. The increase in rate is slight, however, and is limited to about the first week of regeneration. After this time the rate levels off in all series.

Histological study of the thyroid glands of the animals used in these experiments has demonstrated that in all cases in which a definite effect upon tail regeneration was obtained as a result of the treatment, a marked hypertrophy and diminution of colloid had been produced in the thyroid. In cases in which the treatment had been ineffective, the thyroids showed little or no difference from those of control tadpoles. This evidence thus confirms the hypothesis that the effects of thiourea and phenylthiourea upon tail regeneration result from their influence upon the activity of the thyroid gland rather than from any direct effect upon the regenerating tissues.

E. SUMMARY

1. Administration of thiourea, in proper dosage, to tadpoles of *Rana catesbiana* and *Rana pipiens* during the proliferative phase of tail regeneration results in a retardation of the rate of growth of the new tail.

2. The dosage required depends upon the method of administration. Daily injections of .1 cc of thiourea in concentrations as low as .005 per cent were found to be effective in the bullfrog. In the case of administration by solution of the drug in the culture medium, a .05 per cent solution was effective but a .01 per cent solution was ineffective.

3. Administration of thiourea prior to or coincident with tail amputation does not affect the rate of regeneration during the regulative phase.

4. Phenylthiourea causes a retardation of tail regeneration during the proliferative phase in *Rana pipiens* tadpoles when administered by solution in the culture medium in a concentration of .005 per cent.

5. The regeneration rate in young (53 day) and older (81 day) larvae of *Rana pipiens* is affected in the same way and to the same degree by administration of phenylthiourea. The possibility remains, however, that study of larvae of more widely different ages might reveal some differences in response.

6. When *Rana pipiens* tadpoles are subjected to several successive tail amputations the regeneration rate during the early phases of the process is slightly greater after the later amputations than it is after the first. This is true even in tadpoles which are under continuous treatment with thyroid-inhibitors.

7. Histological study of the thyroids of experimental and control animals indicates that the effect of thiourea and phenylthiourea upon tail regeneration is attributable to their influence upon thyroid activity and not to any direct effect upon the regenerating tissues.

8. Preliminary examination of sections of the tails of experimental and control animals indicates that the reduction of thyroxin level in the treated tadpoles not only causes a retardation in growth rate but also has an inhibiting effect upon the differentiation of muscle and notochordal tissue in the regenerating tail. Full confirmation of this observation must, however, await further study.

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